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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Shah et al.

Serial No.: 09/891,983

Filed: June 26, 2001

For: METHODS FOR THE SIMULTANEOUS DETECTION OF HCV ANTIGENS AND HCV ANTIBODIES

Case No.: 6821.US.01

Examiner: Wortman, D.

Group Art Unit: 1648

Certificate of Mailing under 37 CFR §1.8(a): I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to the:

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Alexandria, VA 22313-1450

Date of Deposit: 1-15-04

Kimberly A. Iorio

DECLARATION UNDER 37 C.F.R. § 1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

We, DINESH O. SHAH, GEORGE J. DAWSON, A. SCOTT MUERHOFF, LILY JIANG, ROBIN A. GUTIERREZ, THOMAS P. LEARY, SURESH DESAI AND JAMES L. STEWART, citizens of the United States of America and residents of either Illinois or Wisconsin, do declare and say that:

We are co-inventors of the above-referenced application for patent filed on June 26, 2001.

In the Office Action of April 29, 2003, claims 13 and 14 are rejected under 35 U.S.C. 102(e) as being anticipated by Chien et al. (U.S. Patent Publication No. 2002/0192639

A1). Additionally, claims 13 and 14 are rejected under 35 U.S.C. 102(e) as being anticipated by Bahl et al. (U.S. Patent Publication No. 2003/0049608 A1). Further, claims 13 and 14 are rejected under 35 U.S.C. 102(e) as anticipated by or, in the alternative, under 35 U.S.C. 103(a) as obvious over Aoyagi et al. (U.S. Patent Publication No. 2002/0173493 A1). Additionally, claims 8-11 and 15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Aoyagi et al. (U.S. Patent Publication No. 2002/0173493 A1). Further, claims 8-11 and 15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chien et al. (U.S. Patent Publication No. 2002/0192639 A1). Also, claims 8-12, 14 and 15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bahl et al. (U.S. Patent Publication No. 2003/0049608 A1) in view of Chien et al. (U.S. Patent Publication No. 2002/0192639 A1).

We, conceived and reduced to practice, in the United States, the invention claimed in claims 13 and 14 prior to the priority date (i.e., the date of filing of the provisional application) of Chien et al. (i.e., June 15, 2000), prior to the priority date (i.e., the date of filing of the provisional application) of Bahl et al. (i.e., March 28, 2001) and prior to the filing date of Aoyagi et al. (i.e., April 26, 2002). Further, we conceived and reduced

to practice, in the United States, the invention claimed in claims 8-11 and 15 prior to the filing date of Aoyagi et al. (i.e., April 26, 2002) and prior to the priority date of Chien et al. (i.e., June 15, 2000). Additionally, we conceived and reduced to practice, in the United States, the invention claimed in claims 8-12, 14 and 15 prior to the priority date of Bahl et al. (i.e., March 28, 2001) as well as Chien et al. (i.e., June 15, 2000). These assertions are evidenced by the following:

Attached Exhibit A illustrates that, prior to June 15, 2000 (i.e., the priority date of Chien et al. and the earliest date of the documents cited above), we developed a method for the simultaneous detection of HCV antigens and HCV antibodies in a test sample. In particular, as evidenced by Exhibit A, in one embodiment, the HCV antigens were to be captured on a solid phase, and then the captured antigens were be detected with an antibody (e.g., monoclonal antibody) labeled with a reporter molecule. Further, the solid phase was to be coated with various HCV proteins (e.g., NS3, NS4 and fragments of the core protein) in order to capture HCV antibodies. The antibodies would then be recognized by a second antibody (e.g., goat anti-human IgG) labeled with a reporter molecule.

Further, Exhibit A also illustrates a schematic view of the assay. In particular, the figure establishes how the antibodies in the test sample are to be detected as well as how the core antigens are to be detected using conjugated monoclonal antibodies.

Exhibit B illustrates that prior to the June 15, 2000 priority date of Chien et al., we carried out the assay and obtained positive data. In particular, Exhibit B illustrates various reagents used in the assay (i.e., those coated on the solid phase) and evidences that upon running the assay, results were obtained indicating that one could detect HCV antigen and HCV antibody simultaneously in a sample.

In summary, the attached Exhibits establish that the claimed invention was conceived of and reduced to practice, prior to the priority date of Chien et al. (i.e., June 15, 2000) as well as the subsequent dates of Bahl et al. and Aoyagi et al.

Although all the dates on Exhibits A and B have been blocked out, such dates are prior to June 15, 2000.

We declare further that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that

willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the instant application or any patent issuing thereon.

Respectfully submitted,

1) _____
Dinesh O. Shah
Date: Jan 7, 2004

2) _____
George J. Dawson
Date: Jan 7, 2004

3) _____
A. Scott Muerhoff
Date: Jan 7, 2004

4) _____
Lily Jiang
Date: 1/7/2004

5) _____
Robin A. Gutierrez
Date: 1/9/2004

6) _____
Thomas P. Leary
Date: 01/07/04

7) _____
Suresh Desai
Date: Jan 7, 2004

8) _____
James L. Stewart
Date: 1/8/04

4. ABBOTT LABORATORIES
RESEARCH DEPARTMENT

PROJECT HCV antigen test

EXP. OR CODE NO.

There have been recent indications that HCV core proteins can be detected in serum of HCV infected individuals, most notably the publications from Tonen Corporation (Tanaka et. al, Journal of Hepatology 1995 23: 742-745: and Aoyagi et al., in the Journal of Clinical Microbiology 1999 37:1802-1808. There have been no published disclosures pertaining to an antigen/antibody combo test for detection of exposure to HCV to date. There are several possible methods for devising a combo HCV test, allowing detection of both antibodies and antigens associated with exposure to HCV. Current antigenic targets for the antibody test include recognition of viral proteins derived from several different open reading frames of the virus including core + envelope proteins as well as proteins from nonstructural regions designated as NS (nonstructural) 2, NS3, NS4 and NS5. Commercialized tests currently utilize HCV proteins from NS3, NS4 and/or NS5.

(Continued on page 5)

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RESEARC DEPARTMENT

PROJECT HCV antigen test

EXP. OR CODE NO.

A potential combo test would continue to utilize one or more virally derived proteins from HCV for antibody detection but would also employ antibodies generated against HCV proteins to develop an antigen sandwich assay which captures HCV proteins on a solid phase (microcellulose, microparticles, polystyrene plate or bead) and then further detect the captured protein with a labeled antibody. One of the most likely targets for detection of HCV antigens is the HCV core protein. While Toren corporation has clearly demonstrated utility of an HCV antigen test, there has been no clear indication of a combo test being developed.

For this reason, the following proposal is made - Abbott Labs would develop an antibody/antigen combo test, allowing simultaneous detection of antibodies & antigens associated with exposure to HCV. In one example of this combo assay a solid phase would be coated with HCV protein (NS3, NS4 and fragments of the HCV core protein) and also coated with antibodies to HCV. (continued on page 6)

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WITNESSED BY Lily Fong DATE

ABBOTT LABORATORIES
RESEARCH DEPARTMENT

PROJECT HCV antigen test

EXP. OR CODE NO.

This solid phase would capture antibodies to HCV or would also capture HCV proteins (e.g. Core protein). The captured antibodies (that were captured due to antibodies binding to HCV proteins) would be recognized by a second antibody (e.g. goat anti-human IgG) that is labeled with a reporter molecule (horseradish peroxidase, acridinium, biotin etc). allowing detection of antibodies directed agst the solid phase-bound proteins derived from HCV.

The captured antigens would be recognized in one example of the cmbs assay by specific antibodies (e.g. monoclonal antibodies) against the core protein. This specific antibody would be labeled with a reporter molecule (horseradish peroxidase, biotin, acridinium) to allow detection of the bound antigen.

One of the important differences between the cmbs test for HCV and HIV as proposed here is that one continues to be able to detect antibodies to core and core antigen at the same time. In order to do this successfully, the core protein needs to be re-engineered. See page 7

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RESEARCH DEPARTMENT

PROJECT HCV antigen test

EXP. OR CODE NO.

The core protein of HCV consists of 191 amino acids. For detection of antibodies to HCV only segments of the core molecule would be needed. For example it is known that there are epitope associated with antibody detection at amino acids 9–88 based on literature reviews. Thus, in one version of a combo assay the solid phase would be coated with – NS3, NS4 t/or NS5 proteins and a modified Core protein (containing needed epitopes) as well as one or more monoclonal antibodies (or possibly polyclonal antibodies to core). See Figure on page 7. Further the conjugates would recognize the bound antibodies (captured with specific antigen) or bound antigens (captured with specific antibodies). The candidate core proteins would be: recombinant core proteins (aa 1–100, aa 1–20, aa 8–89, aa 9–99 etc) with monoclonal antibodies recognizing epitopes outside of the sequences recognized by antibodies in human serum. Alternatively one could use peptides 6 mers or greater covering major epitopes between amino acids 1–100. Further the antigens on the solid phase could be re-engineered to include amino acid substitutes, deletes, etc.

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RESEARCH DEF MENT

BOOK NO. 913[illegible]

PROJECT Schematic for Combo test

Schematic of Potential HCV Combo Antibody/Antigen Test

Liquid Phase Conjugates:

Goat anti-human conjugate
- detect antibodies to core, NS3-5

Conjugated mab's to core
- detects core antigen in serum
- conjugates must not recognize solid phase core

Mab's agst aa:
1-8, 89-190, 8-89
(amino acid deletions substitutions are made in core solid phase proteins/peptides)

*Recombinant core aa 8-89 may appear as a single entity or as fragments covering the major epitopes...sequences may be modified by deletions, substitutions between aa 8-89 that do not perturb major epitopes. Synthetic peptides covering aa 8-89 would also be a viable alternative.

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8

ABBOTT LA .ATORIES
RESEARCH DEPARTMENT

BOOK [illegible] 68,160

PROJECT HCV combo Assay Ab, Ag

EXP. OR CODE NO. Blended ingo ind conj.

This is the first demonstration of a combination antibody/Ag test for HCV.

cont. on pge #10

N/A 08/31/00 21:20:16 L JIANG

Blended Up and Blended conjugate

Up: HC31 (DF=3 Coating conc: 200ug/ml) + C11-14 (0.09% 0.4um)
Conjugate: C11-10 (100ng/ml 1:16) + 6A52B (1/5dilution In HIV combo CD)
Washes: HIV ag transfer wash Dev lot 5/ final wash : HCv Ag prep
SDB: 6A52Q
Up diluent: 18498 HCv Ab assay up diluent
S/A configuration: HCV

			Combo Assay		Ab Assay		Ag Assay	
Samples	SubA	SubB	Mean counts	P/N	Mean Counts 08/28/00	P/N	Mean counts 08/24/00	P/N
					33862	8.64		
PC (Ab)	1502	1923	1712.5	2.17	3930.17		79	
NC (Ab)	808	852	780					
	755	745			4618.75	1.23		
	719		719	0.91	36800.67	8.36		
99800	1167	1083	1110	1.41	147307.5	37.46		
Panel A	9785	9035	9410	11.91			4408.83	55.82
E2 1/20 dil	7872	8237	8104.5	10.26			6980.5	88.36
Promed 9992161	2550	3639	3094.5	3.92			681.5	8.63
PC JV 018929	5227	5290	5258.5	6.66	1427	0.36	2645	33.49
PC JV017220	842	899	870.5	1.10	2059.5	0.52	4706.5	59.58
Sero-Tec panel #3	1954	1773	1863.5	2.36	1704.5	0.43	5071	64.19
4	2552	2463	2507.5	3.17	1716.5	0.44	4258	53.92
5	3608	3507	3558.5	4.50	1607.5	0.38	2853	36.11
6	2882	3120	3001	3.80	1671.5	0.43	4829	61.13
7	2055	2290	2172.5	2.75	1665	0.42		
8		3707	3707	4.69				
9								
1277								
21611								

1023 V COMBO ASSAY

As the first Blend up and Conj. results are encouraging. Dilute Conj more for Next run.

SIGNATURE Lily Jiang DATE

WITNESSED BY [illegible] DATE

ABBOTT L' ORATORIES
RESEARCH DEPARTMENT

BOOK NO. 68,160

PROJECT HCV combo Assay

EXP. OR CODE NO. Cont. from page #8.

DESCRIPTION OF PANEL MEMBERS -

NC - negative control - pooled plasma individually screened as negative for HCV antibodies by a commercialized assay- **Code: 6A52E. Prism HCv Ab Assay Negative Calibrator.**
PC - positive control - pooled anti-HCV positive plasma diluted in negative control . **Code: 6A52F. Prism HCV Ab Assay Positive Calibrator.**

99800 - Plasma(human) Recalcified Negative Bulk.

Panel A - an anti-HCV positive plasma that has been diluted in negative control to provide a mid range sample to cutoff in the PRISM antibody assay.

E2 1/20 - an anti-HCV positive sample that has been diluted in negative control - the E2 antibody panel was utilized to titrate the potency of HCV E2 antigen coated microparticles

Promed 9992161 - an antibody positive sample obtained from ProMeDx (Plainville, MA)

PC JV 16929 - Sero-Tec HCV RNA positive human plasma .
PC P JV17220 - Sero-Tec HCV RNA positive human plasma .

SeraTec Panel members 3-9 - serial bleeds obtained from a plasma donor identified at SeraTec as being anti-HCv negative and HCV antigen positive.

A panel of specimens previously characterized as having antibodies to HCV or being negative for antibodies to HCV but positive for HCV RNA and HCV antigens were tested in a preliminary HCV combination antibody.antigen test.

Reagents utilized in combo test
Microparticles specific for HCV antigen detection (up's coated with C11-14 as described on RB: 67093 page 100) and microparticles specific for HCV antibody detection (up's coated with HCV recombinant protein HC 31 as described on RB: 68160page 2) were blended to produce a solid phase that would allow simultaneous detection of HCV antibodies and HCV antigens in a single reaction well. (The blended microparticles contained 0.19% solids, representing a mixture of 0.09% up's coated with C11-14 and 0.1% coated with HC31). The conjugates were also a mixture of two separate acridinium labeled proteins. Acridinium labeled C11-10 was utilized for HCV antigen detection (recognizing HCV antigens captured on the C11-14 microparticles) and an acridinium labeled monoclonal antibodies against biotin -labeled goat anti-human IgG (presented as a pre-complex - see RB: 52226m301) was utilzied to detect human anti-HCV IgG bound to the HC-31 coated microparticles.

Results

The panel described above was run on 3 different PRISM-based assays. One of the assays detected HCV antibodies, a second test detected HCV antigens and a third test (the combo assay) detected both HCV antibodies and HCV antigens.
Sampels have a positive to negative ratio (P/N) ratio of 3.0 or greater were considered positive. The data presented in the table on RB68160page 8 indicate that the combo assay allows detection both of antibody positive samples (e.g. panel E2 1/20, ProMed 9992161, PC JV 016929 and PC JV 17220) and HCV antigen positive samples (Sera Tec panel members 5-9). Thus, this single combo assay performed in a single reaction well detects most of the samples that were positive in two separately performed assays, the HCV antibody test and the HCV antigen test. This is the first demonstration of a combo HCV antibody / HCV antigen test at Abbott Laboratories, and is the first example of the HCV antibody /antigen combo test ideas presented in Redbook 61,959: pages 1-8. Other iterations of the HCV combo test will be presented over the next several weeks/months.

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ABBOTT LABORATORIES
RESEARCH DEPARTMENT

PROJECT HCV combo Assay. *

EXP. OR CODE NO.

Title: HCV combo Assay: Blended up and Blended conjugate

Purpose: To blend the HCV core peptide coated ups, NS3NS4 coated up, c11-14 coated ups together and c11-10 , aHigG Acr* conjugate together for HCV combo first demonstration.

(core peptide Ag + NS3NS4 for Ab Detection)
c11-14 Ab coated up for Ag Detection)

Materials and Samples
RB: 68160001 and 68160011.

Preparation:
Add Avidin 11-28 (df=20) and NS3NS4 (df=10) and c11-14 (0.09% seradyn)
Add conjugate c11-10 (50ng/ml) and aHigG Acr* (10ng/ml)

Results:

HCV Combo (11-28, NS3NS4,c11-14 c11-10, aHigG) 9 12

Conclusions:
The combo assay successfully detected all the Ab pos. samples and Ag positive samples.

Next Steps:
Dilute the AhigG conjugate to 7ng/ml and 2 ng/ml

1023 6 A N/A 09/12/00 14:30:02 L JIANG
HCV COMBO 11-28,NS3NS4
C11-14 C11-10 AHIGG

HCV Combo Assay

Blended ups: HCv Core Bio-11-28(Df=20)+ NS3NS4 HCV Ag (DF=10)+ C11-14(0.09%)
Conjugate: c11-10(5ong/ml) + aHigG Acr*(10ng/ml)
Washes: HCV Ag Assay Transfer: HIV Ag Devlot5, Final wash: HCV Ag final wash prep 8/1/2000
SDB: HCV Ab (6A52Q)
S/A (1023) configuration: HCV

	SubA	SubB	Mean	P/N
PC (Ab)	3454	3656	3555	4.84
PC (Ag)	5303	6014	5658.5	7.71
PC(Ag)	4288	3722	4005	5.46
NC(99B00)	637	831	734	
E2 1/20	12480	13092	12786	17.42
ProMed 9990196	11449			15.60
9990164	15	No conjugate was added		
9990162	10060		10060	13.71
9990212	13925		13925	18.97
Sero-Tec panels #3	956		956	1.30
4	2347		2347	3.20
5	3400		3400	4.63
6	4673		4673	6.37
7	4265		4265	5.81
8	3045		3045	4.15

*Materials: Code/List/Desc/Lot see RB: 68160

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ABBOTT LABORATORIES
RESEARCH DEPARTMENT

BOOK NO. 68.160

PROJECT

EXP. OR CODE NO. Cont. from page #17

1023 6 A N/A 09/15/00 11:10:02 L JIANG
BD SEROCONVERSION

ups 11-28+NS3NS4+C11-14 Conjugate 50ng/ml + 7ng/ml

	SubA	SubB	Mean	P/N
NC	452	456	454.00	
E2 (1/20)	7753	7804	7778.50	17.13
PC (Ag)	4462	4407	4434.50	9.77
9990212	7611		7611.	16.76
9996196	6878		6878	15.15
9996164	5133		5133	11.31
Sero-Tec panel #3	1257		1257	2.77
4	2540		2540	5.61
5	2870		2870	6.32
6	4917		4917	10.83
BBI HCv sero 907 #1	5595		5595	12.32
2	2707		2707	5.96
3	2614		2614	5.76
4	2701		2701	5.95
5	2343		2343	5.16
6	4443		4443	9.79
7	8147		8147	17.94

ups 11-28+NS3NS4+C11-14 Conjugate 50ng/ml + 2ng/ml

	SubA	SubB	Mean	2ng/ml P/N	7ng/ml P/N
NC	277	246	261.50		
E2 (1/20)	2831	2879	2855.00	10.92	17.13
PC (Ag)	4213	4099	4156.00	15.89	9.77
9990212	2773		2773	10.60	16.76
9996196	2249		2249	8.60	15.15
9996164	1918		1918	7.33	11.31
Sero-Tec panel #3	927		927	3.54	2.77
4	2299		2299	8.79	5.61
5	3002		3002	11.48	6.32
6	5112		5112	19.55	10.83
BBI HCv sero 907 #1	3754		3754	14.36	12.32
2	3363		3363	12.86	5.96
3	2230		2230	8.53	5.76
4	2404		2404	9.19	5.95
5	1743		1743	6.67	5.16
6	2084		2084	7.97	9.79
7	1566		1566	5.99	17.94

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BOSTON BIOMEDICA, INC.

Anti-HCV Seroconversion Panel (PHV907)

HCV Genotype 1A

Panel Member	Bleed Date	Day No.	ABBOTT 3.0 HCV S/CO	ORTHO HCV 3.0 S/CO	Combo alt Cont @7 ng/ml S/N	Combo alt Conjug @2 ng/ml S/N	Abbott Ag Only Test S/CO (1)	Roche Amplicor RNA copies/ml
PHV907-1		0	0.1	0.0	12.3	14.4	25.68	3 x 10e6
PHV907-2		4	0.1	0.0	6.0	12.9	20.41	2 x 10e6
PHV907-3		7	0.1	0.0	5.6	6.5	17.88	1 x 10e6
PHV907-4		13	0.2	0.1	6.0	9.2	15.98	1 x 10e6
PHV907-5		18	0.8	0.5	5.2	6.7	6.88	1 x 10e6
PHV907-6		21	1.4	1.0	9.8	8.0	7.90	1 x 10e6
PHV907-7		164	>5.0	>5.0	18.0	6.0	0.70	nd

Data above demonstrates on seven member seroconversion panel, that HCV RNA and HCV Antigens can be detected from the first bleed date (through the sixth bleed date, but the seventh bleed date is negative for HCV antigen. The antibody tests Ortho 3.0 and Abbott 3.0 failed to detect antibodies in the first five bleed dates (through)...

The combo test detected exposure to HCV for all seven bleed dates.

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WITNESSED BY _____ DATE

PROJECT PRISM HCV Ag/Ab combo

EXP. OR CODE NO. HCV combo Assay Random Donor Population

Reagents: Same as Expt # 68160017.

1023 6 A N/A 09/16/00 11:00:17 L JIANG

ONBD SMALL POP

HCV Combo Assay Population

		SubA	SubB	Mean	P/N	Cutoff(3nc)	S/CO	Cutoff (2.5nc)	S/CO	Cut off (2.33nc)	S/CO
PC (Ag)		3737	3854	3796.5	15.30	744	6.10	620	6.12	677.84	6.87
E2 (1/20)		2948	2785	2867	11.66		3.86		4.62		4.98
NC (99800)		214	282	248							
GCRBC plasma #1		182					0.24		0.29		0.31
	2	163					0.22		0.26		0.28
	3	292					0.39		0.47		0.51
	4	172					0.23		0.28		0.30
	5	266					0.36		0.43		0.46
	6	172					0.23		0.28		0.30
	7	271					0.36		0.44		0.47
	8	329					0.44		0.53		0.57
	9	200					0.27		0.32		0.35
	10	247					0.33		0.40		0.43
	11	196					0.26		0.32		0.34
	12	246					0.33		0.40		0.43
	13	432					0.58		0.70		0.75
	14	214					0.29		0.35		0.37
	15	161					0.22		0.26		0.28
	16	215					0.29		0.35		0.37
	17	140					0.19		0.23		0.24
	18	227					0.31		0.37		0.39
	19	284					0.38		0.46		0.49
	20	209					0.28		0.34		0.36
	21	200					0.27		0.32		0.35
	22	185					0.25		0.30		0.32
	23	248					0.33		0.40		0.43
	24	209					0.28		0.34		0.36
	25	291					0.39		0.47		0.50
	26	241					0.32		0.39		0.42

SIGNATURE _____ DATE

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